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Microbiota recovery in a chronosquences of impoverished Cerrado soils with biosolids applications

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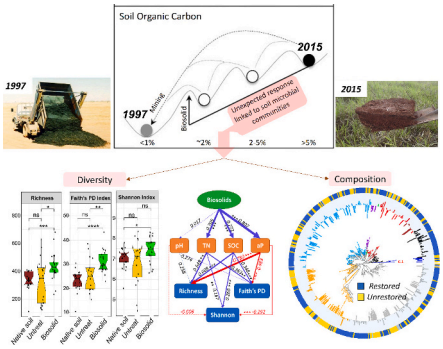
HIGHLIGHTS

- The soil microbiota in a global diversity hotspot Cerrado has been studied
- Soil microbiota showed higher dynamics after mining compared to native soils
- Recovered microbiota with biosolid application benefits carbon enrichment
- Soil carbon and nitrogen greatly contributed to the recovery of microbial diversity

GRAPHICAL ABSTRACT



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ABSTRACT

Mining activities put the Brazilian savannas, a global biodiversity hotspot, in danger of species and soil carbon losses. Experiments employing biosolids have been applied to rejuvenate this degraded ecosystem, but a lingering question yet to be answered is whether the microbiota that inhabits these impoverished soils can be recovered towards its initial steady state after vegetation recovery. Here, we selected an 18-year-old restoration chronosequence of biosolids-treated, untreated mining and native soils to investigate the soil microbiota recovery based on composition, phylogeny, and diversity, as well as the potential factors responsible for ecosystem recovery. Our results revealed that the soil microbiota holds a considerable recovery potential in the degraded Cerrado biome. Biosolids application not only improved soil health, but also led to 41.7 % recovery of the whole microbial community, featuring significantly higher microbiota diversity and enriched groups (e.g., *Firmicutes*) that benefit carbon storage compared to untreated mining and native soils. The recovered community showed significant compositional distinctions from the untreated mining or native soils, rather than phylogenetic

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differences, with physiochemical properties explaining 55 % of the overall community changes. This study advances our understanding of soil microbiota dynamics in response to disturbance and restoration by shedding light on its recovery associated with biosolid application in a degraded biodiverse ecosystem.

1. Introduction

Land degradation due to mining activities causes highly specific and complex threats to the biodiversity and poses substantial challenges for ecological restoration worldwide (Sonter et al., 2018). In tropical regions, which harbor ancient nutrient-depleted soils, mined lands often remain bare for decades following the removal of the topsoil and exposure of infertile substrates (König et al., 2023; Silva et al., 2013). This problem is the case in the Brazilian savanna (known as the 'Cerrado'), which is ranked among the top 35 global biodiversity hotspots and conservation priorities due to its high levels of species endemism (Marchese, 2015; Myers et al., 2000). The Cerrado has been undergoing severe degradation since the 1960s (Rada, 2013), caused by agricultural expansion, urbanization, and mining activities (Beuchle et al., 2015; Sano et al., 2010; Spera et al., 2016). As a result, 46 % of the Cerrado natural area has been occupied by human activities in the last 60 years (Rodrigues Gupta et al., 2022).

In the Cerrado mining region, biosolids are utilized as a source of organic matter and nutrients to restore the original soil physicochemical, biological, and hydrological characteristics (Jordán et al., 2017). This practice has enhanced plant recruitment on rehabilitated soils (Corrêa et al., 2018; de Andrés et al., 2007), thus promoting the structural and functional recovery of the ecosystem (Balduíno et al., 2019; Silva et al., 2015, Silva et al., 2013). However, complex recovery mechanisms that underlie the interactions between the soil physicochemical and biological aspects remain elusive in this impoverished ecosystem. For instant, previous study has revealed the "unprecedented accumulation" of plant-derived carbon in restored soils in this area, which was significantly higher than those found in native savannas in the same region (Silva et al., 2013). That unexpected observation is attributed to the synergistic effects of recovered and/or invasive plant species that colonized the restored sites after applying biosolids, and the subsequent, but yet to be identified microbial stabilization of plant-derived carbon as metallo-organic complexes into soil aggregates (Silva et al., 2015). Even though, recent study has reported that plant microbiota harbor genes involved in organic compound intake in native soils (Camargo et al., 2023), this remains unclear in the rehabilitated soils of this ecosystem.

Despite aforementioned positive effects of such restoration activities on the ecosystem functions (e.g., as a carbon sink), we also lack comparable knowledge on a chronosequence-based monitor on the dynamics of soil microbiota over the recovery process. This is crucial for gaining comprehensive insights into the overall recovery status towards the restoration cycle (Schmid et al., 2020). To improve our fundamental understanding of long-term soil rehabilitation with biosolids applications, we studied the soil microbiota recovery spanning an 18-year-old chronosequence from 1997 to 2015 in biosolids-treated soils, with a direct comparison of untreated mining soils and adjacent soil under natural vegetation. We hypothesized that "unprecedented accumulation" of carbon and nutrients in restored soils had positive effects on microbial diversity and the majority microbial groups, thereby contributing the stabilization of soil organic carbon (SOC) in rehabilitated soil of the Cerrado mining restored area over the time.

2. Materials and methods

2.1. Description of study sites and sampling procedures

Seven time points of post-mining areas that received biosolids in their exposed substrates in 1997, 2002, 2003, 2005, 2008, 2011, and 2015 were selected for this study (Table S1). All mines were located within the Federal District of Brazil, which extends over 5814 km^2 at the Brazilian Central Plateau. (Silva et al., 2015, Silva et al., 2013). The regional topography varies from flat to gently sloped, with an average altitude of 1100 m. The prevailing climate is a tropical savanna (Aw – Köppen-Geiger) with well-defined wet and dry seasons. The mean annual temperature ranges from 21 °C to 24 °C, with annual rainfall ranging from 1200 to 1600 mm (95 % of the precipitation occurs between September and March).

Clay and lateritic gravel were mined in these sites for over 50 years since 1947 prior to the biosolids application in 1997. The mining activities left post-mining substrates from 4 to 5 m below the original surface level. The original soils in these areas were classified as reddish Oxisol and Haplic Inceptisol – *Latosol Vermelho* and *Cambissolo* ("EMBRAPA, 2013), typical soils supporting the Cerrado sensu stricto phytophysiognomy. A total of 100 Mg ha^{-1} of dry biosolids (Table S2) produced through ferric ion precipitation during tertiary sewage waste treatment was used as a common source of OM (~50 % carbon) and nutrients (N, 5.5 %; P, 2 %; K, 0.2 %; Ca, 2.5 %; Mg, 0.5 %; and S, 0.5 %) across sites (Silva et al., 2015). During biosolids production, iron salts ($Fe_2(SO_4)_3$) are used as precipitating and stabilizing agents; thus, the final incorporated biosolids had, on average, about 45 g kg^{-1} of reactive amorphous iron on a mass basis. After incorporating biosolids, the sites were left unmanaged, and natural regeneration with propagules was provided by the surrounding native ecosystems (Silva et al., 2013). Details on the main properties and characteristics of biosolids used in this study are provided in Table S2.

Samples of native soils, biosolids-treated soils, and untreated soils were collected in triplicate in 2015 at all seven sites, calculating 0, 4, 7, 10, 12, 13, and 18 years since the incorporation of biosolids for different sites, respectively (See Table S1). We defined three treatments: untreated mining substrates exposed to the surface (Sub), rehabilitated substrates with biosolids incorporation (Reh), and native soils under Cerrado vegetation (Soil). Each sample consisted of 200 g of material collected with sterilized augers from the top 10 cm of the surface layer. Three replicates were collected at each of the seven study time points, resulting in 21 samples at each treatment. Samples were placed into a styrene made Styrofoam box filled with ice, frozen at −20 °C (<4 h), and transported to the laboratory, where one part was stored at 4 °C for physicochemical analyses, and another part was stored at −80 °C prior to molecular analysis.

2.2. Soil physicochemical analyses

Soil samples stored at 4 °C were sieved (2 mm) and air-dried for soil organic matter (SOM, %) analysis using dry combustion gas chromatography, and the soil organic carbon (SOC) content was calculated via SOM divided by 1.724. The accumulation of SOC over time was presented as the average value of all replicates collected at the study sites. Other analyses followed previous method describe in Huang et al. (Huang et al., 2019). In brief, soil pH (1:1 of soil:water) was measured via potentiometry; total nitrogen (TN, %) was determined using the Kjeldahl method; available phosphorus (aP, mg/kg) was determined using Bray I extraction. Macronutrients (K, Ca, Mg, S) and micronutrients (B, Fe, Mn, Cu, Zn) as well as Al levels were evaluated by Mehlich III extraction (expressed in mg/kg). Cation exchange capacity (CEC, cmol kg^{-1}) was calculated by the summation method of exchangeable cations (Ca, Mg, K, Na, and H).

2.3. Total soil DNA extraction and microbial community profiling

The total soil DNA was extracted in triplicate using 0.5 g of each soil sample as a starting material. The DNA samples were extracted using the FastDNA™ Spin Kit for Soil following the manufacturer's instructions (MP Biomedicals, Irvine, CA, USA). The DNA quality and quantity were checked using agarose gel electrophoresis and the Invitrogen™ Qubit® dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific, Waltham, MA, USA), respectively. The DNA samples were stored at –80 °C until further analysis.

The amplification of the variable region V4 of the 16S rRNA gene was performed using the primer set 515F/806R (Caporaso et al., 2011), designed to include Illumina adapters and 12 bp barcode sequences. Briefly, amplification reactions were carried out in a 20 μL volume containing 10 μL Phusion Hot-Start II High-Fidelity Master Mix (ThermoFisher, Waltham, MA, USA), 1 μL of 1 μM of each primer, 2 μL of 5 ng of the sample DNA, and 6 μL of sterile ddH_2O. Amplicons (PCR products) were pooled in equimolar concentrations (100 ng/each sample) with modified earth microbiome protocol, and sequencing was conducted at the Genome Center UC Davis in the DNA Technologies Sequencing Core using paired-ends (2 × 250 bp) on an Illumina Miseq platform (Illumina Inc., San Diego, CA, USA). A total of 3,075,123 sequences were obtained, ranging from 11,505 to 81,391 reads per sample (an average of 48,811 and a median of 50,817 reads per sample). Raw reads were trimmed to 240 bp for forward reads and 175 for reverse reads, allowing for a maximum of two errors per mate with no ambiguous bases and a Q score ≥ 20 using QIIME 2 (Bolyen et al., 2019). A total of 2,465,059 reads passed quality control, averaging 39,128 reads per sample. In addition, 1,460,270 amplicon sequence variants (ASVs; 23,178 ASVs per sample on average) were obtained after dereplication, read merging, and chimeric filtration using the DADA2 algorithm (Callahan et al., 2016). A tree file of the ASVs was generated via the phylogeny FastTree step. Together with the ASV table and a manually made mapping file, they served as the main inputs for the taxonomy assignment against the SILVA 132 database (Quast et al., 2013).

2.4. Taxonomic and phylogenetic diversity analyses

Prokaryotic taxonomic and phylogenetic diversities were accessed using alpha and beta diversities and calculated using the rarefied ASV table, rooted tree, and mapping file in the R (4.0.2) program. The alpha diversities (richness [observed ASVs], Shannon Index, and Faith's phylogenetic diversity [PD]) were analyzed at even sequence depths for all samples (10,900 reads with Good's coverage >99 %). The beta diversity (nonmetric multidimensional scaling [NMDS]) and the distance to centroid was analyzed based on both Bray–Curtis and weighted Unifrac distances to evaluate the microbial dynamics and differences between different treated soils (including biosolids-treated, untreated mining, and native soils) using the *Phyloseq* (McMurdie and Holmes, 2013) and *Vegan* packages (Gupta et al., 2022). The environmental factors that significantly fit the NMDS model were identified through 999 permutational tests using the *Vegan* package (Gupta et al., 2022).

2.5. Statistical analysis and structural equation modelling

The permutational multivariate analysis of variance (PerMANOVA) was tested for the significance of beta diversity between different treated soils with 999 permutations, and for the explanation (r^2) power of each environmental component on the microbial dissimilarity via the pairwise *Adonis* package in R (4.0.2) (Arbizu, 2023). The analysis of variance (ANOVA) with Tukey's HSD test was used to examine the significant differences in microbial diversity, abundance, and composition (phylum level) among three treated soils (including biosolids-treated, untreated mining, and native soils). Additionally, the Pearson correlation was further deployed to study the relationships between environmental factors and the microbial community at the phylum level for consistency. This was performed using the *ggcor* package in R (4.0.2) (Liaohanpeng, 2023).

To further discern the direct and indirect effects of the top environmental drivers that were identified in PerMANOVA analysis on microbial biodiversity, structural equation modelling (SEM) was deployed to examine the relationships among biosolids, top four environmental factors and microbial diversity using the *Lavaan* R package (Rosseel, 2012). Because we hypothesized that biosolids application would significantly change the soil properties altering microbial diversity, the procedure selected the top four environmental factors with >5 % of the explanation power on the microbial dissimilarity and *P* values of χ^2 test <0.05 in order to indicate the model had significant sufficient fitting. In SEM models, the regression or standardized path coefficient (β) represent the directions and magnitudes of the effects with different treatments.

2.6. Phylogenetic trees and threshold indicator taxa analysis

Phylogenetic trees were constructed for the ASVs that exhibited significant $\log_2$-fold changes after biosolids application in the rehabilitated soils in comparison with the substrate soils, using *FastTree* program (Price et al., 2010) with default parameters and the trees were visualized using the *Phyloseq* and *ggtree* packages in R v.4.0.2 (McMurdie and Holmes, 2013; Yu et al., 2017). The $\log_2$-fold changes of ASVs were analyzed using ANOCOM program developed by Huang and Peddada (2020). Furthermore, we explored indicator genera associated with the biosolids application using the threshold indicator taxa analyses (TITAN) via the *Titan2* package in R (4.0.2) developed by Baker and King (2010). The TITAN method is particularly suitable for soil microorganisms, as communities are known to be species-rich, and many microbial taxa are considered rare. First, the genera >0.01 % in at least of 10 samples were selected and categorized at the genus level. The filtered genera with the taxonomy name and top four contributing environmental factors (>5 %) including: (A) Total nitrogen (TN, 10.32 %), (B) Available phosphorus (aP, 5.04 %), (C) Soil organic carbon (SOC, 6.81 %), (D) pH (7.57 %) in the NMDS model were imported into R (4.0.2) as txt files for the following TITAN analysis with the default setting. The negative (z-) and positive (z+) scores (standardized relative abundance of each taxon to the mean and SD of all samples cross each environmental gradients) obtained from the TITAN analysis indicate the decrease and increase in the relative abundance of each filtered taxon, respectively, in response to a particular environmental gradient.

3. Results

3.1. Biosolids application improved soil quality and facilitated plant colonization

We saw firsthand decreases in the SOC content by 10 times, the TN by 3 times, as well as decreases in macronutrients (i.e., K, S) and micronutrients (i.e., B, Fe, Mn) levels by 2–10 times after 68 years (1947–2015) of mining activity in Cerrado savanna (Table 1). On the contrary, biosolids application improved the soil quality significantly after 18 years of rehabilitation from 1997 to 2015, as seen by the significantly increased SOC, macronutrients (TN, P, K, Ca, Mg) and micronutrients (Fe, Mn, Cu, Zn) in comparison with both native and untreated substrate soils (ANOVA with Tukey's HSD test; $P < 0.05$, $n = 21$; Table 1). Our study also expanded on the improvement of soil quality by demonstrating the subsequent chronological plant colonization (Fig. 1A and B) that were dominated by C_3 plants with overall more herb than wood species (Fig. 1C). In addition, our results highlighted that carbon accumulation in the topsoil (SOC = 6.91 ± 0.64 dag kg^{-1} = 6.91 % ± 0.64 %; Table 1), however, seemed much higher than what we expected in the grassland ecosystem (SOC ~ 2 %; Fig. 1D and F), which matched to the levels of adjacent undisturbed forestry soils (red dash lines in Fig. 1C; SOC > 5 %; Fig. 1E).

Table 1
Changes in soil properties (mean ± standard deviation) of native soil (soil), untreated substrate (Sub) and rehabilitated substrate (Reh).

Soil properties	Soil	Sub	Reh
pH	5.13 ± 0.43 a	5.52 ± 0.45 b	5.66 ± 0.79 b
SOC (dag kg^{-1})	2.12 ± 0.45 a	0.29 ± 0.24 b	6.91 ± 0.64 c
TN (%)	0.18 ± 0.03 a	0.06 ± 0.03 b	0.31 ± 0.15 c
aP (mg/kg)	1.80 ± 0.54 a	4.00 ± 6.06 a	484.64 ± 346.42 b
rP (mg/kg)	25.37 ± 11.52 a	10.15 ± 6.43 b	30.38 ± 12.75 a
K (mg/kg)	84.85 ± 44.46 a	30.96 ± 25.40 b	156.53 ± 163.13 c
S (mg/kg)	27.20 ± 54.11 a	12.70 ± 18.17 b	32.48 ± 28.93 a
Ca (mg/kg)	0.74 ± 0.57 a	0.68 ± 0.87 a	3.79 ± 2.43 b
Mg (mg/kg)	1.10 ± 1.94 a	1.00 ± 2.05 a	1.63 ± 1.90 b
Al (mg/kg)	0.52 ± 0.37 a	0.10 ± 0.00 b	0.33 ± 0.36 c
B (mg/kg)	0.18 ± 0.08 a	0.10 ± 0.00 b	0.24 ± 0.20 a
Zn (mg/kg)	1.67 ± 1.55 a	1.38 ± 0.90 a	26.94 ± 17.36 b
Fe (mg/kg)	85.19 ± 39.82 a	28.13 ± 9.31 b	140.85 ± 99.29 c
Mn (mg/kg)	10.28 ± 3.94 a	1.97 ± 1.33 b	6.73 ± 2.40 c
Cu (mg/kg)	1.43 ± 1.77 a	1.50 ± 1.46 a	3.90 ± 2.62 b
CEC (cmolc/dm^3)	9.44 ± 1.95 a	2.62 ± 1.05 b	19.49 ± 1.95 c

SOC, soil organic carbon; aP, available phosphorus; rP, remaining phosphorus; TN, total nitrogen; CEC, cation exchange capacity. Different letters after numbers indicate significant difference between soils (ANOVA with Tukey's HSD test; $\alpha = 0.05$, $n = 21$).

3.2. The dynamics of microbial community was mainly driven by TN, pH, SOC and aP during the recovery process

We assessed and compared the temporal variation across different soils (biosolids-treated, untreated mining, and native soils) using NMDS plots (Fig. 2A and S1) with calculated distance to centroid (Fig. S2) based on Bray–Curtis and weighted UniFrac distances, respectively. Our results showed a distinct separation of samples in both microbial composition and phylogeny by treatments (Fig. 2A and S1; PerMANOVA, $P < 0.001$; Table S4). In comparison with native soil, both the biosolids-treated and untreated mining soils showed significantly higher dynamics over time based on the analysis of distance to centroid within each soil group (Fig. S2; $P \leq 0.05$ with 999 permutations). We identified 15 out of 22 environmental variables that were significantly linked to these compositional and phylogenetic dynamics (999 permutations, $P < 0.05$; Fig. 2A and S1). Increases in clay content were linked to the community variations observed in the native soil, whereas SOC, macronutrients (TN, P, K, Ca) and micronutrients (Fe, B, Cu, Zn) levels, as well as silt were linked to those changes in the biosolids-treated soil (Fig. 2A and S1). In summary, soil physiochemical properties explained 55 % of the whole community changes, with TN (10.32 %), pH (7.57 %), SOC (6.81 %) and aP (5.04 %) as the top four significant contributing factors (Fig. 2B).

3.3. SOC and TN showed significantly positive effects on microbial diversity

There were significant differences in prokaryotic richness, phylogenetic abundance (Faith's PD index), and Shannon diversity between different treated soils ($P < 0.01$; Fig. 2C). The mean value of richness for the biosolids-treated soils (436.86 ± 99.17; Table S3) was significantly higher ($P < 0.05$) than the values observed in the native soils (334.63 ± 66.05) and untreated soils (341.25 ± 161.62). Accordingly, phylogenetic abundance and Shannon diversity both had a significantly ($P < 0.05$) higher value for samples collected from the biosolids-treated soils compared with those obtained from the untreated soils (Fig. 2C and Table S3). To investigate the physicochemical factors that drive microbial diversity recovery after the edaphic rehabilitation, we employed structural equation modelling (SEM) to examine the relationships among biosolids application, environmental factors (top four were chosen with each >5 % of the explanation power) and microbial diversity. The whole SEM was built with $P < 0.001$ (χ^2 test), and the result showed in Fig. 2D. The SEM illustrated that TN and SOC were significantly and positively affected by biosolids application (standardized path coefficient, $\beta_{TN} = 0.705$, $\beta_{SOC} = 0.772$; $P < 0.001$), and played positive roles in shaping microbial diversity with more effects on compositional than phylogenetic levels ($\beta_{TN\text{-richness}} = 0.549 > \beta_{TN\text{-Faith's pd}} = 0.363$; $\beta_{SOC\text{-richness}} = 0.609 > \beta_{SOC\text{-Faith's pd}} = 0.269$; $P < 0.01$; Fig. 2D). Compared with TN and SOC, aP had an opposite effect on microbial diversity, as presented by the negative path coefficients of $\beta_{aP\text{-richness}} = -0.780$, $\beta_{aP\text{-Faith's pd}} = -0.483$, $\beta_{aP\text{-shannon}} = -0.292$ ($P < 0.001$; Fig. 2D). The pH, however, did not show any significant effects on all three microbial diversity indices in the model.

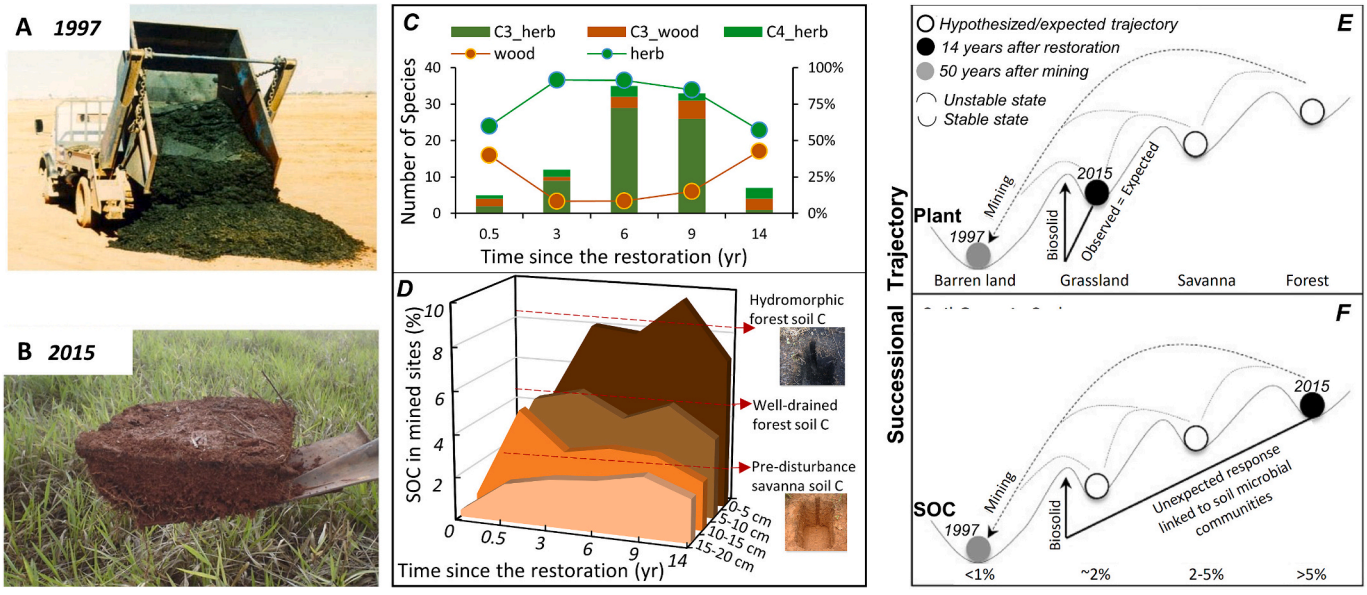

Fig. 1. (A) An abandoned opencast mine with no vegetation cover; (B) a restored mining site with vegetation cover and high soil carbon content; (C) changes in community composition (bars) and relative abundance (dots) of C_3 and C_4 plants at the restored mining site; (D) soil organic carbon accumulation at different soil depths, in comparison with adjacent undisturbed reference soils (red dash lines), following ~14 years of restoration in abandoned opencast mines previously dominated by savannas in central Brazil (adapted from Silva et al., 2013, 2015); (E/F) successional trajectory of plant communities and SOC in the restored mining site over the 18 years of restoration.

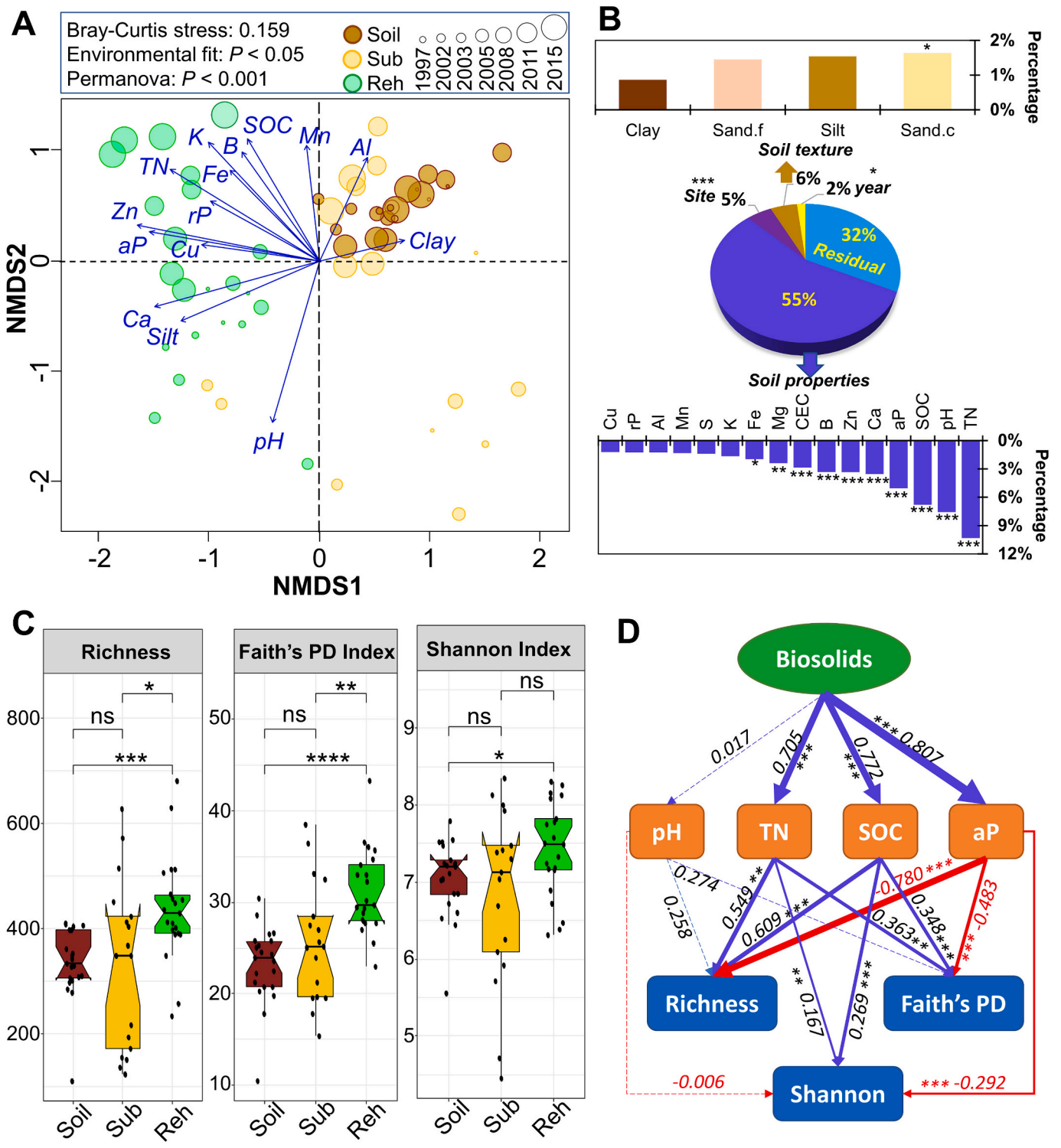

Fig. 2. (A) Nonmetric multidimensional scaling plot based on Bray–Curtis distances of the microbial composition in different soils. Arrows represent an increase in the concentration of the environmental factor with statistical significance at $P \leq 0.05$ after 999 permutations. (B) The explanation (r^2) power of each environmental component on the microbial dissimilarity via Permutational Multivariate Analysis of Variance. (C) Microbial alpha diversities in different soils (richness, Faith's phylogenetic diversity, and Shannon Index). (D) The effects (coefficients on pathway arrows) of biosolid application on the top four contributing factors as shown in (B) and microbial diversity in (C) using structural equation modelling (SEM). Blue and red arrows indicate positive and negative relationships, respectively. Solid or dashed lines indicate significant or non-significant relationships. Soil, native soil (brown); Sub, untreated substrate (gold); Reh, rehabilitated substrate (green). SOC, soil organic carbon; aP, available phosphorus; rP, remaining phosphorus; TN, total nitrogen; CEC, cation exchange capacity. Sand.f, fine sand; Sand.c, coarse sand. Symbols indicate the significance at $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***).

3.4. Biosolids application affected the composition of majority microbiota

The effects of biosolids on microbial composition varied considerably among different microbial lineages at different levels. At phylum level, we observed significant shifts in 10 of the 23 shared phyla (> 95 % of total community) among these soils (Fig. 3A). When compared to native soil samples, biosolids application significantly increased the relative abundances of *Actinobacteria* ($P < 0.01$), *Firmicutes* ($P < 0.001$),

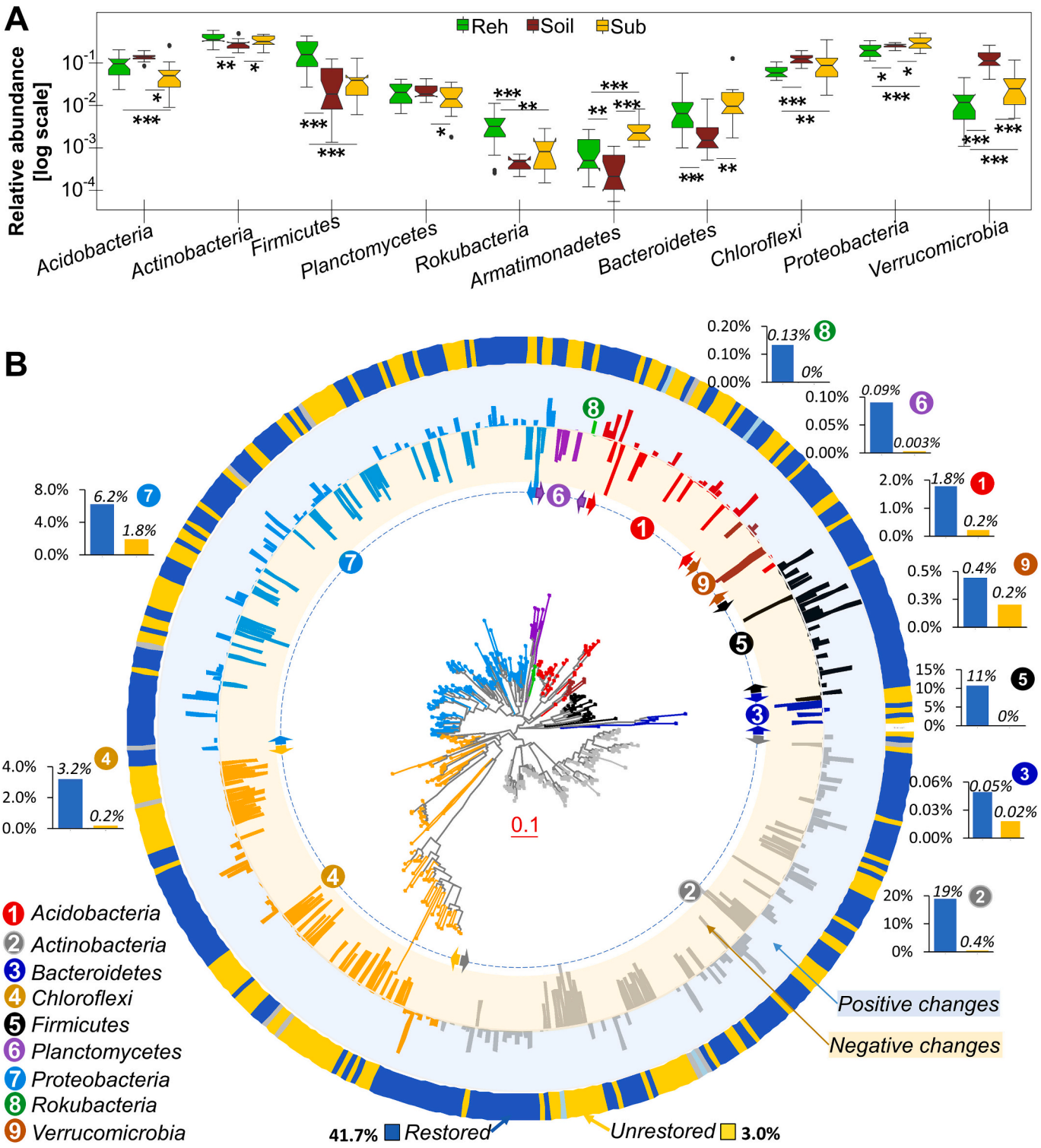

Fig. 3. (A) The difference of microbial composition at phylum level among different soils; (B) The phylogenetic tree of bacterial ASVs (reads ≥2 in at least 5 samples) with a significant response to biosolid application. The bars of the second ring represent the positive and negative changes (log 2-fold changes at $P < 0.05$) in Reh soil compared to Sub soil. Heatmap ring represents either restored (blue) or unrestored ASVs (yellow) after biosolid application in Reh soil in comparison with native soil. The bar plots with numbers outside the whole tree show the total relative abundance of ASVs in each phylum that are restored (blue) and unrestored (yellow) after biosolid application in Reh soil, respectively. Soil, native soil (brown); Sub, untreated substrate (gold); Reh, rehabilitated substrate (green). Symbols indicate significance at $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***).

and *Rokubacteria* ($P < 0.001$), with concomitant decrease in the relative abundances of *Proterobacteria* ($P < 0.001$), *Chloroflexi* ($P < 0.01$) and *Verrucomicrobia* ($P < 0.001$) (Fig. 3A). Meanwhile, these 10 phyla were also shown significant correlations with soil physicochemical properties (Fig. 4). In particularly, *Firmicutes* had significant positive correlations with most of the soil physicochemical properties ($P < 0.01$; Table S5), except for pH; while *Proterobacteria*, *Chloroflexi* and *Verrucomicrobia* were significantly negatively correlated with most of the soil properties ($P < 0.05$; Table S5). In addition, we investigated the changes in taxonomic groups with significant response to the biosolids application (log_2-fold changes at $P < 0.05$) at fine taxonomical levels using individual amplicon sequence variants (ASVs; Fig. 3B). The results suggested that *Actinobacteria* group harbored the highest proportion ASVs, with 19 %, that significantly and positively responded to the biosolids application, followed by the order of *Firmicutes* (11 %) > *Proterobacteria* (6.2 %) > *Chloroflexi* (3.2 %) > *Actinobacteria* (1.8 %) > *Verrucomicrobia* (0.4 %) > *Rokubacteria* (0.13 %) > *Plantomycetes* (0.09 %) > *Bacteroidetes* (0.05 %). In general, 41.7 % of them were restored or even increased above the original level, and only 3 % were still unrestored after biosolids application in comparison with native soil.

3.5. Indicator genera associated with the biosolids application

We further analyzed the indicator genera related to the environmental changes due to biosolids application using the TITAN algorithm. We identified more significant indicator genera (30–50) associated with the changes in soil TN, aP, and SOC gradients comparing with the changes in pH gradient (Fig. 5 and S5). In general, the negative indicator genera took up 0.15–3.09 % of the whole community in average, which was ~10 times lower than that of positive indicator genera (15.44–26.77 % in average) in these soils. Of all the significant indicator taxa, *Nitrolancea* (*Chloroflexi*) was identified as the only positive responder with highest changing points of both TN (0.42 %; Fig. 5A) and aP (759 mg/kg; Fig. 5B) at 95 % confidence interval; *Bacillus* (*Firmicutes*) was identified as the only and the most abundant genera (5.27 %) that significantly and positively responded to all four factors with lower changing points of 0.06 % for TN (Fig. 5A), 14.5 mg/kg for aP (Fig. 5B), 0.46 dag kg^{-1} for SOC (Fig. 5D), and 5.3 for pH (Fig. 5D), comparing to other indicator genera in the *Firmicutes*. Altogether, our results suggested that the environmental changing points of microbiota varied considerably among different microbial groups and even within different microbial lineages in the same group, which consequently affected the recovery status of these groups with biosolids application.

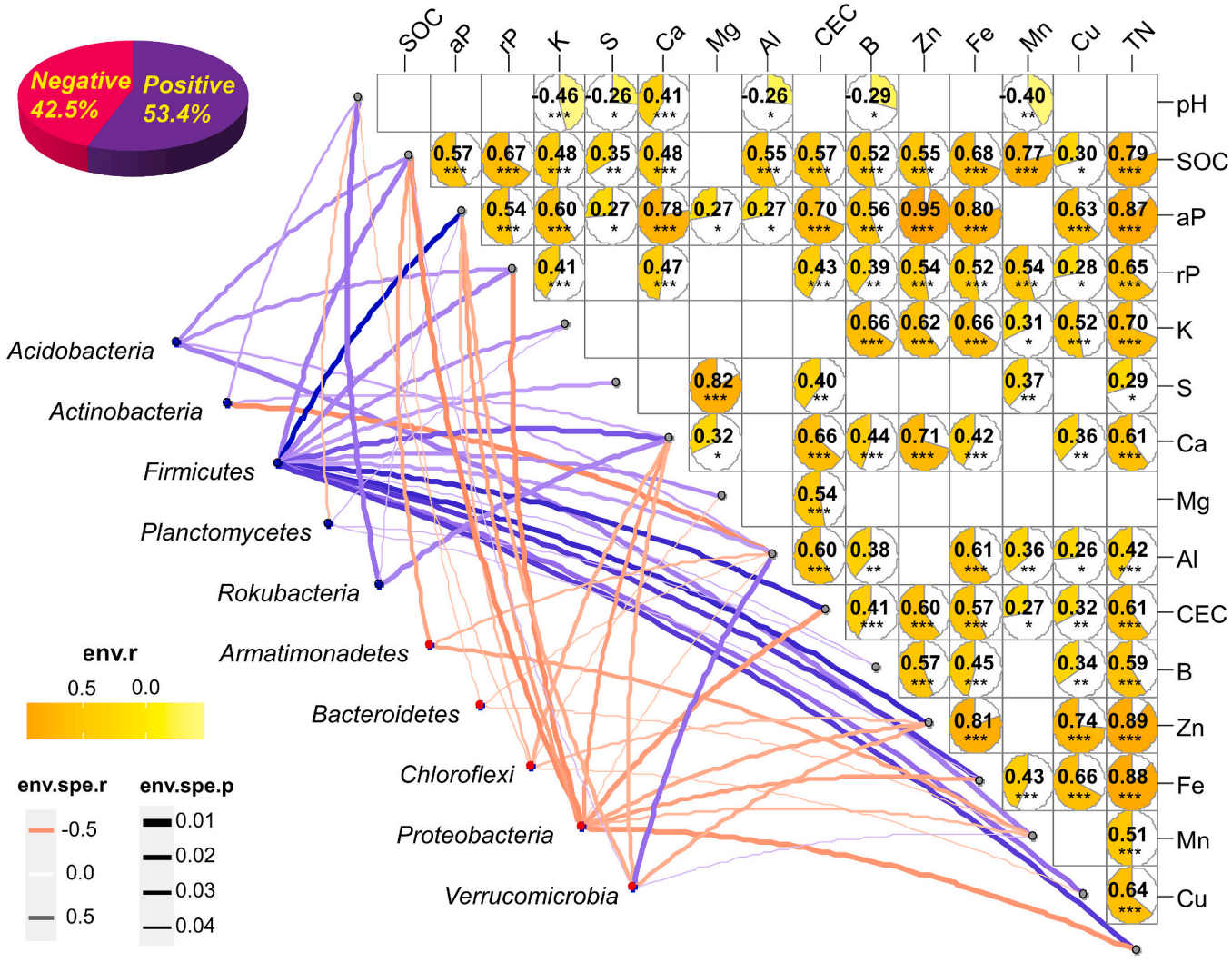

Fig. 4. Pearson correlation coefficients between environmental factors and microbial community groups at phylum level. SOC, soil organic carbon; aP, available phosphorus; rP, remaining phosphorus; TN, total nitrogen; CEC, cation exchange capacity. env.r, the Pearson correlation coefficient between environmental factors; env.spe. r/p, the Pearson correlation coefficient/*p* values between environmental factors and species (phylum level). Symbols indicate significance at $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***).

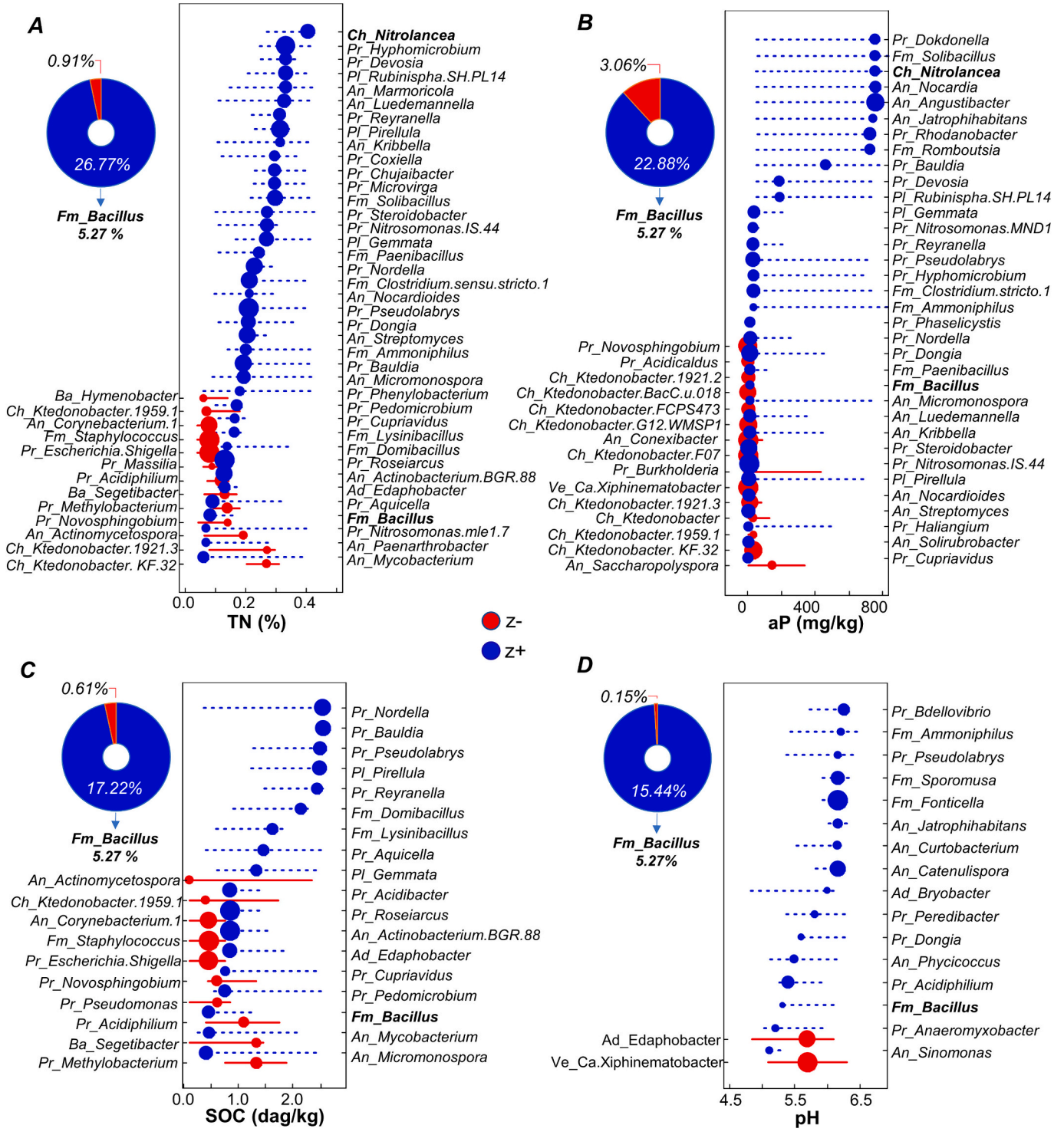

Fig. 5. Threshold Indicator Taxa Analysis (TITAN) of the genera (> 0.01 % in at least of 10 samples) response to the top four contributing factors (>5 %) including: (A) total nitrogen (TN, 10.32 %), (B) available phosphorus (aP, 5.04 %), (C) soil organic carbon (SOC, 6.81 %), (D) pH (7.57 %). Only significant indicator taxa (purity >0.95, reliability>0.95, *p*-value <0.05) are plotted in these Fig.s. Blue symbols represent positive (z+) indicator taxa; red represent negative (z-) indicator taxa. Symbol sizes are proportional to z scores. Horizontal lines overlapping each symbol represent the 5th and 95th percentiles for 500 bootstraps with 21 soil replicates. The first two letters represent phylum name: Ac, *Acidobateria*; An, *Actinobacteria*; Ba, *Bacteroidetes*; Ch, *Chloroflexi*; Fm, *Firmicutes*; Pl, *Planctomycetes*; Pr, *Proteobacteria*; Ve, *Verrucomicrobia.*

4. Discussion

4.1. Changes in microbial diversity and composition over the course of recovery

Upon comparison with native soil, we notice that the untreated mining soils harbored comparable levels of alpha diversity (refer to Fig. 2C). We assume that this similarity may be attributed to the legacy effects of the relic DNA. This type of DNA has been found to be more likely present in soils with low exchangeable base cation concentrations (CEC), affecting microbial community structure more profoundly in high-pH soils (Carini et al., 2016). In Cerrado sites, we have low pH soils (<5.7) with a CEC value of 2.62 ± 1.05 (untreated soil), 9.44 ± 1.95 (native soil), and19.49 ± 1.95 (biosolids-treated soil; see Table 1). In this case, it is most likely that relic DNA will have a greater influence on untreated soil that had low CEC, potentially leading to an increase in the diversity of live microbiota. Overall, biosolids-treated soils exhibited increased microbial diversity (Fig. 2C) and higher levels of soil micro- and macro- nutrients (Table 1), fostering a more diverse plant community (Fig. 1) compared to both native soil and untreated soil. These findings collectively suggest an improvement of soil health through the application of biosolids (Bhatia, 2008; Fierer et al., 2021; Gupta et al., 2022; Lehmann et al., 2020; Nielsen et al., 2002). However, we found that the increased microbial diversity (Fig. 2C) was not exactly reflected in the increased compositional similarity (Fig. 2A). Existing studies suggest that the composition of most microbial groups may not exhibit resilience to disturbance at least within a few years globally (Allison and Martiny, 2008; Lem Gupta et al., 2022) or even half a century (Schmid et al., 2020). A previous study on the soil microbiota recovery under iron-mining under revegetation also supported this concept (Vieira et al., 2018), suggesting a strong vegetation selection on the soil microbiota recovery in their study.

Meanwhile, the sampling design in this study allowed us to assess the influence of the time of the biosolids application on the recovery of the soil microbiota. The relative abundance of some microbiota showed small fluctuations for approximately 4 to 7 years after the exposed to biosolids, but a clear decrease occurred beyond this time limit. This decrease was exemplified by the analysis of the phyla *Acidobacteria* and *Verrucomicrobia*, with an explicit decrease in the relative abundances over the time (Fig. S4). The dominance of these two phyla in Cerrado soils (Araujo et al., 2012; Camargo et al., 2023; de Castro et al., 2016; de Castro et al., 2013) has been recognized elsewhere. The loss of *Acidobacteria* should be of concern, given that this group has excellent metabolic versatility involved in regulating carbon, nitrogen, and sulfur cycles and the potential to produce a diverse range of secondary metabolites (Eichorst et al., 2018; Hill et al., 2011; Kalam et al., 2020). In the same breath, the phylum *Verrucomicrobia*, with few cultured representatives (compared with other phyla, e.g., *Proteobacteria*), is considered oligotrophic and sensitive to a high nutrient supply of carbon and nitrogen in soil (Aguirre-von-Wobeser et al., 2018; Ranjan et al., 2015). This phylum plays a particular important role in Cerrado ecosystem functioning, where it contributes nitrogen fixation (Camargo et al., 2023) and controls on CH_4 oxidation (Dunfield et al., 2007) and CO_2 production (Wertz et al., 2012), as well as N_2O emissions (Mohammadi et al., 2017) in soil ecosystems.

4.2. The effects of SOC on microbiota recovery

Carbon pool mostly predominantly resides in terrestrial soil ecosystems (Torri et al., 2014) and is identified as one of the most important factor among all measured soil properties that determine the recovery status of the peatland microbiota (Emsens et al., 2020). In our study, we identified SOC as the most predominant positive factor contributing the increases in the microbial diversity over the recovery periods with more significant effects on compositional rather than phylogenetic level ($\beta_{SOC\text{-}richness} = 0.609 > \beta_{SOC\text{-}Faith's\ pd} = 0.269$; $P < 0.01$; Fig. 2D). Meanwhile, certain specific groups observed in our biosolids-treated soils benefited from the increases in the SOC storage. Notably, *Actinobacteria* and *Firmicutes* group, with the most abundant ASVs (19 %, 11 %) among all the phyla, exhibited significantly and positively responses to the biosolids application. Moreover, these groups have been shown to play important roles in soil organic matter protection. *Actinobacteria* facilitate the soil C storage by enhancing soil aggregate formation via producing hyphae (Barka et al., 2015; Kieser et al., 2000), while *Firmicutes* contribute to this process through the production of extracellular polymeric substances, respectively (Costa et al., 2018). Of particular significance, *Bacillus (*in *Firmicutes* group*)* was observed as the most abundant genera (5.27 %) of the whole community and showed significant response to the increased gradients of SOC in our study (Fig. 5), which have been identified one of the best genera with highest aggregation potentials (Costa et al., 2018). Microbially induced aggregate formation has long been identified as a critical mechanism of soil carbon stabilization (Martin et al., 1955) that have been extensively studied in agricultural soils (Gupta et al., 2022; Six et al., 2002).

4.3. Self-recovery of microbiota in untreated soil

The soil microbiota from the untreated mining soil showed much higher similarity in diversity, composition, and phylogeny in comparison with the native soils after 68 years (1947–2015) of mining activity than that from biosolids-treated samples with 18 years (1997–2015) of rehabilitation (Fig. 2 and S1). This suggests that the soil microbiota was in a process, albeit slow, of self-recovery over half a century after mining activity. In line with the recent studies, self-recovery of soil microbiota after stress removal have been reported in a degraded peatland ecosystem induced by draining activities in Europe (Emsens et al., 2020) and in a degraded coastal wetland ecosystem by reclamation activities in China (Huang et al., 2020) at a large scale. Both studies reported a substantial soil microbiota recovery at compositional, phylogenetic, and functional levels after stress removal. Particularly, it is important to point out that soil microbiota demonstrated the same recovery potential under bauxite mine via returning topsoil to mining sites in Western Australia's jarrah forest across a 28-year post-mining rehabilitation chronosequence (Peddle et al., 2023).

The high similarity between the untreated mining soils and the native Cerrado soils is noteworthy after 68 years of mining activity with two critical implications. First, some microorganisms in the Cerrado might display traits associated with resistance, the degree to which a community endures disturbance (Pimm, 1984; Shade, 2018; Shade et al., 2012a). For instance, *Firmicutes* did not show any significant fluctuations over the time in both untreated mining and native soils with similar relative abundance at the end (Fig. S4). Members of this group are known to form spores (Galperin, 2016), to be resistant to desiccation and extreme environmental conditions (Teixeira et al., 2010). Meanwhile, soils under Cerrado *stricto* sensu are well-drained, deep, and poorly fertile (Haridasan, 2008). This ecosystem, recognized for its limited nutrient availability, is known for maintaining oligotrophic microbial groups resistant to acidic and nutrient limited conditions. Like the *Acidobacteria* group (Kalam et al., 2020; Macrae et al., 2012), which was kept relatively stable for almost 10 years after 2005 in both untreated mining and native soils. Second, any disturbance, such as mining, would alter the microbial community as an unintended consequence. This alteration might be maximal to a certain degree at the beginning, but as the time passed after stress removal, substantial recovery of microbiota may occur in these ecosystems (Allison and Martiny, 2008; Emsens et al., 2020; Huang et al., 2020; Shade et al., 2012a, 2012b).

5. Conclusions

Through a comparative analysis of soil microbiota in a biosolids-treated rehabilitation site with untreated and natural savanna sites

across an 18-year chronosequence, we provided empirical evidence that soil microbiota exhibited a higher recovery potential at phylogenetical than compositional levels in both biosolids-treated and untreated sites following post-mining rehabilitation. In comparison with the soil microbiota via biosolids rehabilitation, our results further indicated that the soil microbiota in the untreated mining soil via natural self-recovery after 68 years (1947–2015) of mining activities appeared to be more similar to natural savanna sites at both compositional and phylogenetic levels, suggesting a strong sign of natural resilience of soil microbiota. Despite of that, biosolids application improved the soil health, increased microbiota biodiversity, as well as gathered some specific groups like *Firmicutes* that was suggested to potentially facilitate the carbon restoration co-benefits in this ecosystem in a short term of recovery period (i.e., 18 years of biosolids rehabilitation vs. 68 years of self-recovery). Future research incorporating a multiomics approach could help providing more clues to understanding the carbon accumulation process via this group in Cerrado open-cast mining restoration area.

CRediT authorship contribution statement

Laibin Huang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Alexandre Soares Rosado:** Writing – review & editing, Resources, Funding acquisition. **Alonna Wright:** Writing – review & editing, Resources. **Rodrigo Studart Corrêa:** Writing – review & editing, Resources. **Lucas Silva:** Writing – review & editing, Supervision, Resources, Conceptualization. **Jorge L. Mazza Rodrigues:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interest.

Data availability

All data are available in the NCBI Sequence Read Archive under the accession number PRJNA718342.

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Appendix A. Supplementary data

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